Policy Brief

An Updated Look at Mancozeb:
Concerns about Farmworker Reproductive and Child Developmental Health

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Executive summary

Mancozeb is a broad-spectrum fungicide commonly used in agriculture worldwide. It is a member of the ethylene bisdithiocarbamate (EBDC) group of fungicides, which includes the related active ingredients maneb and metiram. Currently, exposure to mancozeb is considered to pose minimal human health risks by most regulatory bodies. Despite mounting scientific evidence, the potential adverse reproductive and human health developmental effects of mancozeb, especially in sensitive subpopulations (e.g., pregnant women, children, and agricultural workers), has not yet been fully considered in the registration and reregistration of this widely used fungicide.

We urge the EPA to systematically evaluate and consider the reproductive, pregnancy, and fetal health impacts of the fungicide mancozeb in its upcoming Registration Review. In particular, we urge the Agency to consider the full risks – including developmental and reproductive risks - from exposure to mancozeb experienced by vulnerable populations, such as all children under the age of 21 and women who work in agricultural operations (including in fields, nurseries, and ferneries) and who work with agro-chemicals. The planned scheduled opening of the Registration Review docket for mancozeb is the third quarter of FY2015.

We make this request because current EPA policy does not reflect acknowledged data gaps and uncertainty about the health impacts of mancozeb. Our systematic review of mancozeb research indicates that the fungicide presents a risk of both reproductive and developmental harm to humans. Furthermore, recent cases of infants born with severe birth defects to farmworkers possibly exposed to mancozeb in violation of regulations should trigger the EPA to act in a precautionary manner by taking action to address the risk concerns from the use of mancozeb.

The Problem

Our research with farmworkers of childbearing age demonstrates that: (1) an agricultural work environment characterized by multiple stressors, including pesticide exposure, prolonged elevated temperatures, and musculoskeletal strain, can influence reproductive health, and (2) the farmworker community is profoundly concerned about the health risks associated with agricultural work, the scarcity of guidelines, information or regulations protecting the health of pregnant women in the workforce, and (3) the uncertainty regarding the impact of these exposures on pregnancy and child health.[1,2] One chemical that has been of particular concern in the farmworker community is mancozeb.

A systematic review of the relevant scientific literature related to mancozeb is provided in Appendix A. Mancozeb is an ethylene bisdithiocarbamate (EBDC) fungicide with the degradate ethylenethiourea (ETU). ETU has known teratogenic, mutagenic, and carcinogenic risks. The State of California and the Toxic Release Inventory (TRI) lists mancozeb as a carcinogen. The TRI also lists mancozeb as a developmental and reproductive toxicant. One study in rodents has reported that mancozeb and ETU are capable of crossing the

[1]Flocks J, Kelley M, Economos J, McCauley L. Female farmworkers' perceptions of pesticide exposure and pregnancy health. *J Immigr Minor Health* 2012:14: 626-632.

[2] Flocks J, Thien Mac V, Runkle J, Tovar-Aguilar JA, Economos J, McCauley L. Female farmworkers' perceptions of heat-related illness and pregnancy health. *J Agromedicine* 2013:18: 350-358.

placental barrier and can damage DNA and initiate tumors in fetal cells.[3] Mancozeb is also a suspected endocrine disruptor and is associated with both hyperthyroidism and hypothyroidism.[3a,b]

From 2004-2006, six infants were born with a range of birth defects to farmworkers in southwest Florida. Of these, three cases were investigated by the Centers for Disease Control and Prevention in collaboration with state health departments in Florida and North Carolina (the two states in which the mothers worked harvesting tomatoes for the same agricultural company). In a report on the cases, mancozeb and ETU were implicated because in two cases it could be shown that the pregnant mothers had been exposed to the fungicide in violation of Restricted Entry Intervals. Also, the specific birth defects of the farmworkers' infants resembled those observed in laboratory studies of rats exposed to mancozeb and ETU.[4] This is concerning since our research with female farmworkers in Central Florida revealed higher urinary metabolite levels of ETU compared with non-detect levels of ETU in other nationally representative women and Mexican Americans reported in the National Health and Nutrition Examination Survey (NHANES), suggesting the risk to women and their babies may be much more widespread than just these documented cases in Florida.[5]

It is not just pregnant women and developing fetuses that are especially sensitive to pesticides, but all workers under the age of 21. Puberty is recognized as a critical or sensitive window of susceptibility to chemicals, [6] particularly with respect to reproductive and endocrine disrupting effects. For example, exposure of rodents to high doses of some phthalates has been shown to delay onset of puberty while pubertal dosing of two rat species with the phthalate DEHP has been shown to delay onset of puberty and cause testicular damage[7] and exposure to various chemicals can delay or accelerate onset of puberty in females.[8] Data primarily from laboratory animal and cell culture studies have revealed more than 50 pesticides that have known or suspected endocrine disrupting properties on the female reproductive system.[9,10] These risks of reproductive harm are not fully addressed in EPA's mancozeb registration process.

We recognize that for farmworkers to be adequately protected against occupational hazards, such as pesticides,

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[3] Shukla Y, Arora A. Transplacental carcinogenic potential of the carbamate fungicide mancozeb. *J Environ Pathol Toxicol Oncol* 2001:20: 127–131.

[3a] Goldner WS, Sandler DP, Yu F, Hoppin JA, Kamel F, and Levan TD. Pesticide use and thyroid disease among women in the Agricultural Health Study. *Am J Epidemiol* 2010: 171 (4): 455-64.

[3b] Axelstad M, Boberg J, Nellemann C, et al. Exposure to widely used fungicide mancozeb causes thyroid hormone disruption in rat dams but no behavioral effects in the offspring. *Tox Sci.* 2011: 120 (2): 439-46.

[4] Calvert GM, Alarcon WA, Chelminski A, et al. Case report: three farmworkers who gave birth to infants with birth defects closely grouped in time and place—Florida and North Carolina, 2004–2005. *Environ Health Perspect* 2007:115: 787–791.

[5] Runkle JD, Tovar-Aguilar JA, Economos E, Flocks J, Williams B, Muniz J, Semple M, and McCauley L. Pesticide risk perception and biomarkers of exposure in Florida female farmworkers. *J Occup Environ Med* 2013:55: 1286-92.

[6] Woodruff TJ, Carlson A, Schwartz JM, Giudice LC. Proceedings of the summit on environmental challenges to reproductive health and fertility: executive summary. *Fertil Steril* 2008:89: 281-300.

[7] Noriega NC, Howdeshell KL, Furr J, Lambright CR, Wilson VS, Gray LE Jr. Pubertal administration of DEHP delays puberty, suppresses testosterone production, and inhibits reproductive tract development in male Sprague-Dawley and Long-Evans rats. *Toxicol Sci* 2009:111: 163-78.

[8] Woodruff TJ, Carlson A, Schwartz JM, Giudice LC. Proceedings of the summit on environmental challenges to reproductive health and fertility: executive summary. *Fertil Steril* 2008:89: 281-300.

[9] Bretveld RW, Thomas CMG, Scheepers PTJ, Zielhuis GA, Roeleveld N. Pesticide exposure: the hormonal function of the female reproductive system disrupted? *Reprod Biol Endocrinol* 2006:4: 30.

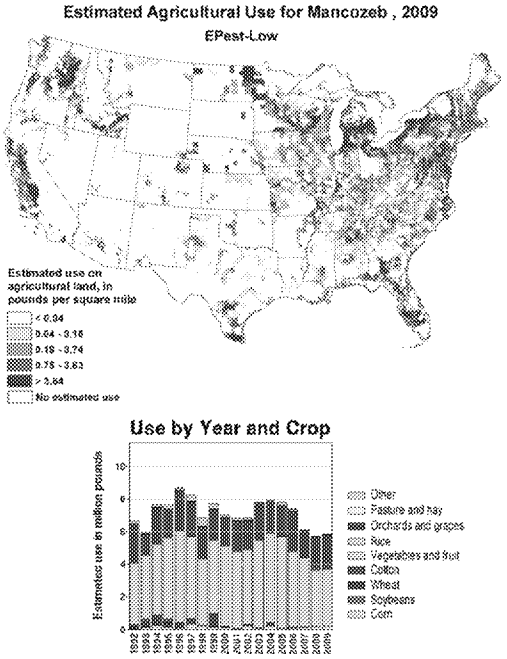
[10] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocr Rev* 2009:30: 293-342.

there must be meaningful regulation that is adequately and uniformly enforced. At present, pesticide regulations are often ineffective or unenforced. Furthermore, existing regulations are often not based upon consideration of the reproductive health impacts and developmental outcomes, particularly for chronically exposed and vulnerable populations such as farmworkers and their children. Accurate exposure and risk characterization of mancozeb as a potential reproductive toxicant is increasingly becoming a priority in the scientific community, yet regulatory bodies are lagging in their consideration of the potential reproductive health impacts in the reassessment of the currently registered uses of mancozeb. EPA must consider the growing evidence of human health harm in its upcoming Registration Review docket for mancozeb in 2015.

Regulatory History and Current Policy

According to the 2005 Reregistration Eligibility Decision (RED),[11] mancozeb was first registered in the United States in 1948 as a broad spectrum fungicide for use in agriculture, turf management, and horticulture. The Mancozeb Task Force was formed in 1994 to represent the interests of the mancozeb registrants, which were then two companies: Rhom and Haas and E.I. DuPont De Nemours. The 2005 RED states that at that time the registrants included Dow AgroSciences, Griffin (now a DuPont subsidiary), and Cerexagri (RED p. 3)

Mancozeb has demonstrated 50 years of fungicidal efficacy for roughly 400 different plant pathogens. It is commonly used on a variety of crops including tree fruits, vegetable crops, field crops, ornamental plants, and sod farms. In 2005, the EPA reported that approximately 5.6 million pounds of mancozeb were used annually. The United States Geological Survey map and chart below show the estimated amount and distribution of agricultural use for mancozeb in 2009, the last year with confirmed crop data from the Census of Agriculture, and a breakdown of crops on which mancozeb was used. As the map shows, mancozeb use is particularly heavy in Florida and North Carolina.



Source: U.S. Geological Survey, *Pesticide Use Maps – Mancozeb*, http://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2011&map=MANCOZEB&hilo=L&disp=Mancozeb. (Accessed May 23, 2014).

The 2005 RED states that mancozeb and other EBDCs have been the subject of two special EPA reviews,

[11] EPA 2005. Reregistration Eligibility Decision for mancozeb. Office of Prevention, Pesticides, and Toxic Substances. EPA 738-R-04-012

initiated in 1977 and 1987, because of particular health concerns, including developmental and thyroid effects, caused by their common degradate ETU. According to the RED, the final determination of the second special review concluded that the dietary risks for EBDCs exceeded the economic benefits for 11 fruit and vegetable food/feed crops, resulting in the cancellation of the use of mancozeb and other EBDCs on these specific crops (RED pp. 2-3). The conclusion of the 2005 RED was to allow for continued use of mancozeb provided that certain risk mitigation measures were adopted and labels amended to reflect those measures. Certain uses of mancozeb, however, were voluntarily canceled, including its foliar use on cotton, use on residential lawns/turf, and use on athletic fields. In the RED, the EPA raised several risk concerns and recognized data gaps for developmental, reproductive, and thyroid toxicity, including:

- Risk estimates were not provided for the following scenarios due to lack of worker exposure data: applicator using liquid dip for seed-piece treatment, applicator using dusts for commercial or on farm seed treatment, and secondary handling for hand planting treated seed pieces. These data will be required in the DCI for this RED (RED p. 44)
- EPA intends to issue a DCI requiring submission of additional environmental fate data for mancozeb parent, the mancozeb complex, and the ETU degradate (RED p. 53)

These above studies would likely be very relevant to an evaluation of the exposures and resultant health risks to workers, including women and children in agriculture fields. It is not clear whether or not EPA ever received these data from the registrants, and, if received, what the data concluded.

In addition to the studies above, the following guideline studies were identified as missing, and required as part of a DCI (RED pp. 101-102):

Toxicology:
870.6200 Acute neurotoxicity
Occupational Exposure
875.1100 Dermal exposure monitoring, outdoor (potato seed piece treatment with liquids and dusts)
875.1300 Inhalation exposure monitoring, outdoor (potato seed piece treatment with liquids and dusts)
Toxicology
870.3700 Developmental toxicity study in rabbits
870.3800 2 Generation Reproductive Toxicity Study
870.6300 Developmental neurotoxicity study
Special study Comparative thyroid toxicity study in young and adult rats

The RED states that EPA "intends to issue a separate product-specific data call-in (PDCI) outlining specific data requirements" (RED p. 103). These above studies are directly and critically relevant to an evaluation of the exposures and resultant health risks to women and children – including underage workers - in agriculture fields. It is not clear whether or not EPA ever received these data from the registrants, and, if received, what the data concluded regarding developmental and reproductive risks to humans from mancozeb.

Since 2005, the EPA has issued final rules on tolerances for residues of mancozeb on various crops including almonds, cabbage, lettuce, peppers, and broccoli in 2011[12] and walnuts and tangerines in 2013.[13] In these final

[12] Mancozeb; Pesticide Tolerances. 40 CFR Part 180. 2013 ed.
[13] Mancozeb; Pesticide Tolerances. 40 CFR Part 180. 2011 ed.

rules, EPA continued to acknowledge certain gaps for mancozeb and ETU, especially regarding impact on the developing thyroid and reproductive systems.

The 2013 final rule acknowledged that maternal mortality, clinical signs, spontaneous abortion, thyroid effects, body weight gain decrements, and decreased pup body weight were observed in rat and rabbit studies with mancozeb. It acknowledged fetal malformations, hydrocephaly, and domed head were observed in rat and rabbit studies with ETU.

For mancozeb and ETU, the EPA retained the 10X FQPA safety factor for women of childbearing age and for children less than six years old. Yet, despite the data gap and uncertainty, the EPA determined that children older than six would be adequately protected if the FQPA safety factor were reduced to 1X, even though these are the years of puberty in which hormonal disruption could render children and youth most vulnerable. It is not clear how EPA made that determination, given the substantial data gaps and lack of relevant information, along with scientific evidence that pregnant women and children under age 21 exposed occupationally to pesticides represent vulnerable and inadequately-protected populations.

For vulnerable populations, such as farmworkers, who are more frequently and intensely exposed to agrochemicals, the risk associated with data gaps and uncertainties is multiplicative. The EPA has seemingly recognized these inherent risks for farmworkers. In the 2005 RED, the EPA stated: *"Based on its evaluation of mancozeb, the Agency has determined that mancozeb products, unless labeled and used as specified in this document, would present risks inconsistent with FIFRA. Accordingly, should a registrant fail to implement any of the risk mitigation measures identified in this document, the Agency may take regulatory action to address the risk concerns from the use of mancozeb."* It is not clear to us whether or not the registrants fully implemented the label requirements to mitigate risk, or if not, whether any Agency regulatory action was taken.

Systematic Review of Research on Mancozeb

The evidence summarized in our review of thirty-six studies suggests that mancozeb presents a risk of both reproductive and developmental harm to humans. Data from *in vitro* studies provide *strong evidence* that mancozeb may indirectly disrupt or impair reproduction at the cellular level and should be regarded as a reproductive toxicant. Animal studies also provide *strong evidence* that mancozeb may induce reproductive and developmental toxicity in mammals. A summary of the data is below, and is presented in more detail in the systematic review in *Appendix A*.

- *In vitro* studies provide strong evidence in support of mancozeb as a reproductive toxicant, with over a decade of research demonstrating high endocrine disrupting potential, significant cytotoxic and pro-apoptotic patterns at 10 and 50 ug/ml between 24 and 48 hours, developmental injury in murine pre-implementation embryos, oxidative and genotoxic damage associated with chronic exposure, premalignant status in somatic cells of mammalian ovarian follicles, and dose-dependent anti-androgenic effects likely involved in fetal disruption of male sexual development.
- Early *in vivo* studies failed to find a significant effect on reproduction for female rats exposed to the maximum tolerated dose of ETU before and during gestation (80 mg/kg/day); however, *significant morphological fetal abnormalities, with the fetal brain most commonly affected, were detected at doses showing no maternal toxicity or fetal mortality in rat offspring*.
- Several studies documented a reduction in reproductive potential in female animals at higher levels of exposure, while mixture studies revealed that prenatal exposure to a low dose pesticide mixture containing mancozeb may lead to adverse developmental toxicity in F1 generations including disruptions in the developing mouse cerebellar cortex, long-term delayed effects at dose levels where single pesticides showed no effects, and genital malformations in male rat pups.

- Animal studies also confirmed reproductive and developmental toxicity in mammals and suggested significant changes in physiological, biochemical, and pathological processes involved in normal reproduction may lead to infertility in males chronically exposed to mancozeb.
- Epidemiological studies examining the effect of mancozeb on reproduction were mixed, but results suggested that mancozeb may interfere with normal reproductive functioning of women persistently exposed (either occupationally or indirectly by spouse's occupation) and thereby may delay or complicate pregnancy.
- Few population-based studies have examined the association between exposure to mancozeb and adverse reproductive or developmental outcomes.

Policy Recommendations

In summary, *high confidence ratings* from *in vitro* and animal studies, in combination with *moderate confidence ratings* from human health studies, provide sufficient evidence that mancozeb should be regarded as a *presumed* developmental and reproductive hazard in humans. We urge the EPA to systematically consider the reproductive, pregnancy, and developmental health impacts of mancozeb. The chronic exposure experienced in vulnerable populations, such as farmworkers and children, to mancozeb makes this matter urgent. The planned scheduled opening of the Registration Review docket for mancozeb is the third quarter of FY2015.

The EPA has previously acknowledged data gaps in its consideration of mancozeb toxicity. To address this, the Agency should clearly identify the data for which it issued a DCI and when it expected to receive that data, a check-list of the studies that were submitted to EPA in response to the DCI, a link to the Data Evaluation Record (DER), and a short summary of what the data results were. EPA should furthermore indicate if there are any outstanding studies that have not been received, or that were received but were found to be unacceptable as guideline studies.

In light of the acknowledged data gaps, we urge the agency to reconsider the 1X FQPA safety factor for children over six. The inconsistent safety factor pattern makes little sense in the face of continuing uncertainties about the health impacts of mancozeb and ETU, especially for vulnerable populations. Currently, girls and boys ages 6-13 receive a 1X safety factor, whereas girls and women ages 13-49 receive a 10X safety factor. Yet, children under age 21 should not be evaluated as adults. Puberty is recognized as a critical or sensitive window of susceptibility to chemicals [14] particularly with respect to reproductive and endocrine disrupting effects. For example, exposure of rodents to high doses of some phthalates has been shown to delay onset of puberty while pubertal dosing of two rat species with the phthalate DEHP has been shown to delay onset of puberty and cause testicular damage[15] and exposure to various chemicals can delay or accelerate onset of puberty in females.[16] Data primarily from laboratory animal and cell culture studies have shown over 50 pesticides to have known or suspected endocrine disrupting properties on the female reproductive system.[17,18] Studies have reported that

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[14] Woodruff TJ, Carlson A, Schwartz JM, Giudice LC. Proceedings of the summit on environmental challenges to reproductive health and fertility: executive summary. *Fertil Steril* 2008:89: 281-300.

[15] Noriega NC, Howdeshell KL, Furr J, Lambright CR, Wilson VS, Gray LE Jr. Pubertal administration of DEHP delays puberty, suppresses testosterone production, and inhibits reproductive tract development in male. Sprague-Dawley and Long-Evans rats. *Toxicol Sci* 2009:111: 163-78.

[16] Woodruff TJ, Carlson A, Schwartz JM, Giudice LC. Proceedings of the summit on environmental challenges to reproductive health and fertility: executive summary. *Fertil Steril* 2008:89: 281-300.

[17] Bretveld RW, Thomas CMG, Scheepers PTJ, Zielhuis GA, Roeleveld N. Pesticide exposure: the hormonal function of the female reproductive system disrupted? *Reprod Biol Endocrinol* 2006:4: 30.

[18] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocr Rev* 2009:30: 293-342.

dioxin causes abnormal breast development in pre-pubertal girls, the plastics-component Bisphenol A (BPA) has shown to cause precocious puberty in girls, the pesticide endosulfan has been reported to affect pubertal boys by slowing down the timing of reproductive maturation, and the fungicides vinclozolin and iprodione have been shown in pubertal male rats to impair normal puberty hormone levels.[19,20,21,22] EPA has not adequately addressed the potential risks for reproductive harm from childhood exposures to mancozeb. Until this is done, the full 10X FQPA factor should be retained for all children, male and female, under the age of 21.

EPA should also consider the occupational health of vulnerable populations, such as farmworkers in the case of mancozeb, as part of its risk assessments. Farmworkers experience multiple stressors in addition to pesticides, including poverty, lack of sanitation, heat stress from outdoor field work, lack of access to health care, poor housing conditions, risk of pesticide drift into farmworker housing, lack of stability of home life, separation from family, rape and sexual assault risks, and other significant issues. Because of economic instability, it is not uncommon for farmworker women to continue working while pregnant, sometimes well into the third trimester and our research has indicated that there are no special workplace accommodations made for pregnant farmworkers, who risk direct contact to pesticide residues or drift.[23] Furthermore, some low-income farmworkers do not have access to childcare, and farmworkers have been known to bring their children to the workplace with them. Farmworkers are paid by a piece rate system and the resulting production demands often means they do not have or take the time to practice occupational safety behavior such as frequent handwashing, wearing adequate protective clothing, or replacing defective equipment.[24,25] Sometimes workers are not provided with proper PPE and/or decontamination supplies by their employer and must provide it themselves.[26] Despite federal regulations, farmworkers often lack access to basic information about pesticides applied at their worksites. Widespread lack of compliance and inadequate enforcement of current Worker Protection Standards means that many farmworkers receive no or minimal pesticide health and safety training or that information about pesticides applied is not being posted correctly.[27] Language barriers can impede the understanding of workers of the risks posed by the pesticides around which they are working, including those who mix, handle, and/or apply the pesticides. Pesticide labels are generally in English only. Even in the best scenarios in which

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[19] Cowin PA, Gold E, Aleksova J, O'Bryan MK, Foster PM, Scott HS, Risbridger GP. Vinclozolin exposure *in utero* induces postpubertal prostatitis and reduces sperm production via a reversible hormone-regulated mechanism. *Endocrinology* 2010: 151:783-92.

[20] Blystone CR, Lambright CS, Cardon MC, Furr J, Rider CV, Hartig PC, Wilson VS, Gray LE Jr. Cumulative and antagonistic effects of a mixture of the antiandrogens vinclozolin and iprodione in the pubertal male rat. *Toxicol Sci* 2009:111:179-88.

[21] Roy JR, Chakraborty S, Chakraborty TR. Estrogen-like endocrine disrupting chemicals affecting puberty in humans--a review. *Med Sci Monit* 2009:15:RA137-45.

[22] Schoeters G, Den Hond E, Dhooge W, van Larebeke N, Leijs M. Endocrine disruptors and abnormalities of pubertal development. *Basic Clin Pharmacol Toxicol* 2008:102:168-75.

[23] Runkle JD, Tovar-Aguilar JA, Economos E, Flocks J, Williams B, Muniz J, Semple M, and McCauley L. Pesticide risk perception and biomarkers of exposure in Florida female farmworkers. *J Occup Environ Med* 2013:55: 1286-92.

[24] Mayer B, Flocks J, and Monaghan P. The role of employers and supervisors in promoting pesticide safety behavior among Florida farmworkers. *Am J Ind Med* 2010:53:814-24.

[25] Runkle JD, Tovar-Aguilar JA, Economos E, Flocks J, Williams B, Muniz J, Semple M, and McCauley L. Pesticide risk perception and biomarkers of exposure in Florida female farmworkers. *J Occup Environ Med* 2013:55: 1286-92.

[26] Flocks J, Kelley M, Economos J, McCauley L. Female farmworkers' perceptions of pesticide exposure and pregnancy health. *J Immigr Minor Health* 2012:14: 626-32.

[27] Flocks J, Monaghan P, Albrecht S, Bahena A. Florida farmworkers' perceptions and lay knowledge of occupational pesticides. *J Commun Health* 2007:32:181-94.

an employer conscientiously complies with all existing regulations, current WPS training requirements do not require information about reproductive health or risks posed by take-home exposures, although this is being considered under the proposed regulations currently open for public comment.

In 2009, the Committee on Improving Risk Analysis Approaches Used by the U.S. EPA, part of the National Research Council (NRC), emphasized the need for cumulative risk assessments, as defined by EPA, "that include combined risks posed by aggregate exposure to multiple agents or stressors; aggregate exposure includes all routes, pathways, and sources of exposure to a given agent or stressor. Chemical, biologic, radiologic, physical, and psychologic stressors are considered in this definition."[28] The NRC report points out that, "although cumulative risk assessment has been used in various contexts, there has been little consideration of nonchemical stressors, vulnerability, and background risk factors" and recommends EPA seriously consider adopting the recommendations of a NRC report on phthalates[29] that "addresses issues related to the framework within which dose-response assessment can be conducted in the context of simultaneous exposures to multiple stressors." The NRC report also emphasizes that "variability in human susceptibility has not received sufficient or consistent attention in many EPA health risk assessments… greater attention to susceptibility in practice is needed, particularly for specific population groups that may have greater susceptibility because of their age, ethnicity, or socioeconomic status."[30] EPA should implement the recommendations of these NRC reports with mancozeb and other pesticide assessments.

Finally, more detail is needed for EPA's assessment of endocrine effects of mancozeb. EPA should describe how the agency will move forward on its analysis of endocrine disrupting effects and specifically address how it considers non-linear dose responses and adverse health effects occurring at extremely low doses (e.g. parts per trillion) for which there is a growing body of scientific evidence. For example, pesticides, at extremely low levels, can interfere with the proper functioning of hormone systems including estrogen, androgens, thyroid, and the pituitary/hypothalamic axis. When exposures, even at very low levels, coincide with critical windows of development, they may be more potent than higher dose exposures, or the low dose exposure may otherwise exert a non-traditional dose-response curve. The consequences of exposure to an endocrine disruptor may also be delayed with additive or synergistic effects from a myriad of chemical exposures. Data primarily from laboratory animal and cell culture studies have shown that more than 50 pesticides have known or suspected endocrine disrupting properties on the female reproductive system.[31,32]

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[28] National Research Council. *Science and Decisions: Advancing Risk Assessment.* Washington, DC: The National Academies Press, 2009, 478 pages.

[29] National Research Council. *Phthalates and Cumulative Risk Assessment: The Task Ahead.* Washington, DC: The National Academies Press, 2008, 208 pages.

[30] National Research Council. *Science and Decisions: Advancing Risk Assessment.* Washington, DC: The National Academies Press, 2009, 478 pages.

[31] Bretveld RW, Thomas CMG, Scheepers PTJ, Zielhuis GA, Roeleveld N. Pesticide exposure: the hormonal function of the female reproductive system disrupted? *Reprod Biol Endocrinol* 2006:4: 30.

[32] Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, Zoeller RT, Gore AC. Endocrine-disrupting chemicals: An endocrine society scientific statement. *Endocr Rev* 2009:30: 293-342.

Appendix A: Systematic Review Findings

In Vitro Studies. Seven *in vitro* studies published between 2000 and 2012 met our inclusion criteria (Figure 1, Table 1). We found seven *in vitro* studies in which the reproductive toxicity of mancozeb was examined. The media for most of these studies was mammalian cells. All of these studies provide evidence that mancozeb may indirectly disrupt or impair reproduction at the cellular level.

Lin et al.[1] showed mancozeb induced cytotoxicity and apoptotic patterns of cell death at doses of 10 and 50 ug/ml between 24 and 48 hours of exposure, while ETU produced apoptotic induction at much higher concentrations (1000 ug/ml). A later study examining adrenocortical steriodogenic cells of rainbow trout demonstrated significant impairment of cortisol secretion at low-doses (50 ug/ml) and showed mancozeb as the highest ranked endocrine disruptor based on LC50/EC50 values.[2] Mancozeb exposure did not result in increased embryotoxicity or a higher incidence in developmental abnormality in chicken embryos, but when mixed with copper sulfate showed significant embryomortality compared to individual doses.[3] Another mixture study revealed that single exposure to mancozeb induced developmental injury in murine pre-implemenation embryos and a fungicide mixture (including mancozeb) reduced blastocyst development and increased apoptosis.[4] Calviello et al.[5] studied the DNA damaging and proapoptotic effects of mancozeb in RAT-1 fibroblasts and peripheral blood mononucleated cells (PBMC) isolated from Wistar rats at one and four hours post-exposure. Following one hour of exposure to 500 ug/ml of mancozeb, fibroblast cells produced dose-dependent induction of single strand breaks and elevated levels of DNA oxidation and reactive oxygen studies. After four hours, mancozeb induced dose-dependent apoptosis in fibroblasts and PBMC cells.

Finally, two recent studies demonstrate mancozeb should be regarded as a reproductive and developmental toxicant. Paro et al. examined the toxic effects of mancozeb exposure on granulosa cells from mice and women undergoing assisted reproductive procedures.[6] Granulosa cells from mice exposed to mancozeb showed time- and dose-dependent changes in morphology, an acquired ability to migrate, decreased levels of p53, but no changes in apoptosis. Human granulosa cells showed dose-dependent morphology changes and reduced p53 expression post-exposure to mancozeb. This study demonstrates that mancozeb damages the somatic cells of mammalian ovarian follicles by inducing a premalignant-like status in both mice and human granulosa cells. In 2013, Kjeldsen et al.[7] demonstrated the *in vitro* effects of mancozeb on the androgen receptor (AR) function in human breast carcinoma MVLN cells and hamster ovary CHO-K1 cells. Results showed concentration-dependent effects on the agonist-induced AR transactivity, with mancozeb showing the highest inhibitory effect, suggesting a link between mancozeb as an AR disruptor and disorders of male sexual health. Because mancozeb has been shown to cross-placental barriers in mice, it is likely that mancozeb can disrupt fetal reproductive development in males.

Overall, in vitro studies provide strong evidence that mancozeb is a reproductive or endocrine disrupting xenobiotic.

Animal Studies. Fifteen experimental studies in mice, rats, rabbits, and pigeons published between 1973 and 2012 met our inclusion criteria (Figure 1, Table 2). In all fifteen studies, mancozeb was either administered orally in food or delivered directly into the stomach (gavage) for lengths of time ranging from 30 days to 1 or more years. While each study included in the review measured individual exposure to mancozeb, several studies simultaneously examined exposure to other chemical agents including: maneb, probineb, endosulfan, and phosphamidon. Four studies assessed independent exposure to mancozeb and then as a mixture with copper oxycloride (one study), paraquat (one study), and procymidone, epoxicanazole, tebuconazole, and prochloraz (two studies). Duration of exposure and dose ranges in female studies (twelve studies) were categorized as: (1) 15 to 30 *before gestational days* (lowest dose: 500 mg/kg/day and highest dose: 800 mg/kg/day); (2) 1 to 30

gestational days (lowest dose: 5 mg/kg/day and highest dose: 1,300 mg/kg/day); and (3) *postnatal days* (lowest dose: 6.25 mg/kg/day and highest dose: 500 mg/kg/day). For studies examining only males (n=3), length of experiment time ranged from 5 to 360 days, with daily doses ranging from 10 to 1,500 mg/kg. Details are below.

Reproductive & developmental endpoints

Females. Two early experimental studies dating back to the 1970s examined the teratogenic effects of mancozeb. Khera investigated prenatal survival and teratogenicity in rats and rabbits.[8] Results indicated no significant effect on reproduction for female rats exposed to the maximum tolerated dose of ETU before and during gestation (80 mg/kg/day). *However, significant morphological fetal abnormalities, with the fetal brain most commonly affected, were detected at doses showing no maternal toxicity or fetal mortality in rat offspring*. While increased resorption sites and reduced brain weight at 80 mg/kg/day were noted for female rabbits, rabbit offspring showed no sensitivity to mancozeb at any dose level and no teratogenic effects on developing brains. Larsson et al.[9] reported profound internal and skeletal malformations in rat offspring. For the highest level of mancozeb exposure (1330 mg/kg/day), the frequency of internal malformations was markedly higher than that of external malformations. The low-dose group (380 mg/kg/day) resulted in significant reductions in fetal weight for exposed mice. Following a twenty-year gap, a collection of animal studies examined the effect of mancozeb exposure on reproductive and pregnancy health in female rats with mixed results. While an earlier study[10] showed no change in the number of estrous cycles post-mancozeb exposure, several studies documented a reduction in the number of estrous cycles, duration of proestrus, estrus, and metestus, healthy follicles and number of corpora lutea with greater than 600 mg/kg/day mancozeb treatment (700 mg/kg/day and 800 mg/kg/day).[11-13] A significant increase in thyroid weight was also observed in all rats treated with mancozeb, except for the group treated with 500 mg/kg/d.[11,12] High prenatal doses of mancozeb (500 mg/kg) resulted in reduced maturity and fertilizability of oocytes in females of the F1 generation.[14] These studies mark the first report on the effects of mancozeb on reproductive potential in animals.

Whole animal studies examining prenatal exposure to mancozeb report significant adverse effects. Axelstad et al.[15] observed toxic effects in rat dams treated with high doses of mancozeb (150 mg/kg/d) including severe weight loss and temporary paralysis in hind limbs. Results also showed a dose-dependent decline in T4 levels for all dams exposed to mancozeb (50 mg/kg, 100 mg/kg, and 150 mg/kg) on gestation day fifteen. Four mixture studies included in the review revealed that prenatal exposure to a low dose pesticide mixture containing mancozeb may lead to adverse developmental toxicity including disruptions in the developing mouse cerebellar cortex[16], long-term delayed effects at dose levels where single pesticides showed no effects[17], and genital malformations in male rat pups.[18]

Males. Experimental studies showed significant declines in male reproductive functioning including, spermatoxic effects in mice, and biochemical and structural alterations in testes of rats and mice following chronic exposure. Khan & Sinha[19] observed decreased sperm counts and significantly higher sperm head abnormalities in all three groups exposed to mancozeb (3, 6, or 1,000 mg/kg/day). Abnormal spermatic head size occurred most frequently in mice treated with mancozeb. A long-term experimental study[20] in male rats resulted in higher mortality-related acute effects in all groups fed mancozeb. During months 5 through 12, male rats demonstrated prolonged reproductive effects at higher doses of mancozeb (1,000 and 1,500 mg/kg/d) including a small increase in testes weight and reduction in epididymis size. Animals in the high dose group (1,500 mg/kg/d) demonstrated temporal changes in pathology for seminiferous tubules, with pronounced damage in epithelial cells of epididymis resulting in reduced sperm count. Ksheerasagar & Kaliwal[21] also found time-dependent declines in weight of testes and other organs (liver, kidneys, and spleen), as well as, significant increases in thyroid and thymus weight of treated mice.

Animal studies provide strong evidence for acute toxicity in mammals and suggest significant changes in physiological, biochemical, and pathological processes involved in normal reproduction may lead to infertility in males chronically exposed to mancozeb.

Human Health Studies. Fourteen epidemiologic studies published between 2001 and 2007 met our inclusion criteria (Figure 1, Table 3.) The majority of studies included used a case-control (six studies) and cross-sectional design (four studies), with the exception of one case study and three cohort studies (one prospective study and two retrospective studies). We observed substantial differences in mancozeb exposure assessment. Most studies assessed exposure indirectly, using pesticide use questionnaires or database registries (eleven studies). Three studies examining Italian vineyard workers obtained individual measures of recent mancozeb exposure by measuring urinary metabolite levels of ETU.[22-24]

Pre-conception. Studies examining the fertility effects of mancozeb or other members of the ethylene bisdithiocarbamate (EBDC) group of fungicides, which includes the related active ingredients maneb and metiram, were mixed. Farr et al.[25] found that women who reported using mancozeb or maneb were four times more likely to experience longer menstrual cycles and two times more likely to have a missed a menstrual cycle compared to unexposed women living on farms in Iowa or North Carolina. In a case study of three women working in similar agricultural industries around the same time, two of the women were exposed to mancozeb during the first trimester, and all three women gave birth to infants with severe malformations.

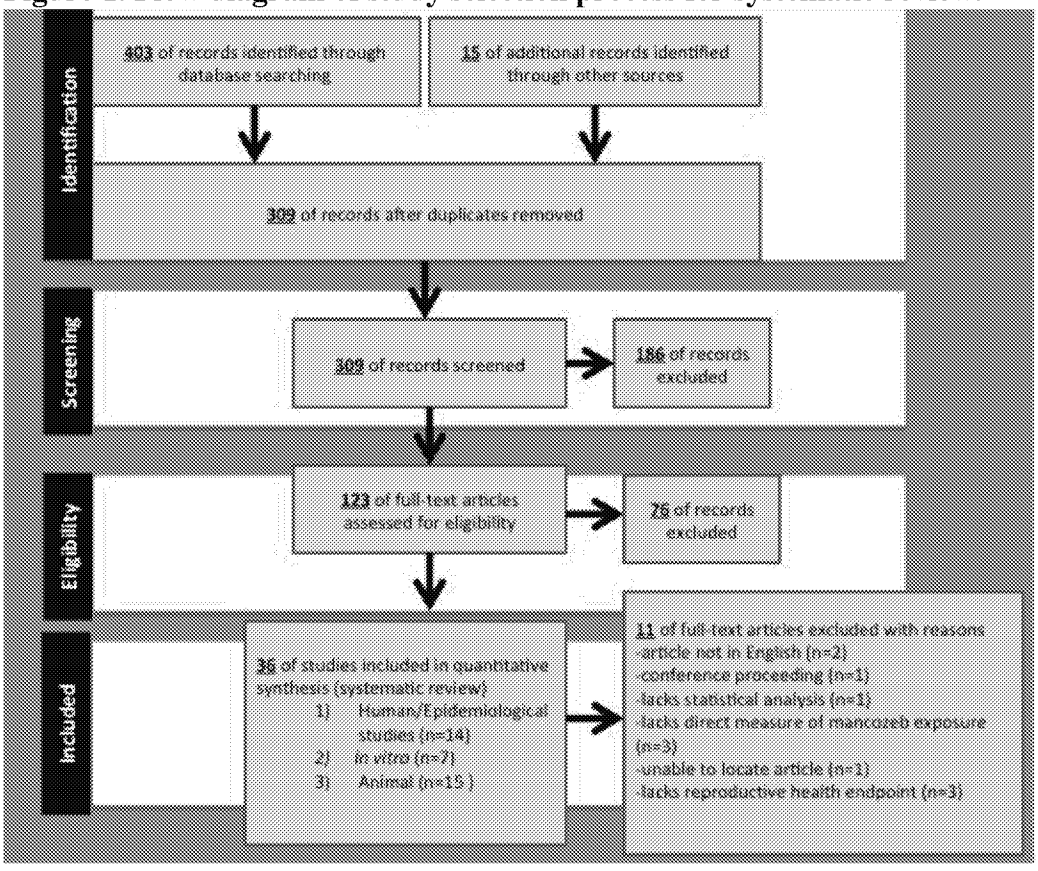
Mancozeb is considered an endocrine disruptor. While Rull et al. observed no apparent effect on neural tube defects when mancozeb was applied within 1,000 meters of maternal residences, authors found an increased odds ratio of 1.5 and 2.0 of having a baby with a neural tube defect or anencephaly, respectively, among pregnant women living within 1,000 meters of where two or more endocrine disrupting chemicals were applied.[26]

Some studies report on the fertility impacts of fungicide exposures generally, but do not identify which fungicides the subjects were exposed to. A trend in longer time to pregnancy was observed in female flower production workers exposed to fungicides year-round.[27] In an early study, Arbuckle et al. observed elevated risk of late-term spontaneous abortions among farm couples exposed to fungicides and double the risks of spontaneous abortions in older women. [28] A later study demonstrated no difference in birth rate for families of fungicide applicators compared to applicators using herbicides and other agrochemicals (2.85 vs 2.80). Garry et al.[29] examined spouses of pesticide applicators in the Red River Valley and found an association between personal use of pesticides and an increased risk of fetal loss. This study also observed an increased frequency of miscarriages for summer, fall, and winter combined among spouses of pesticide applicators who worked with fungicides.

A modest risk of fetal death during the first and second trimesters, especially the 3rd and 4th month of pregnancy, was found in women exposed to thiocarbamates during pregnancy.[30] In this study, mancozeb was assigned to the carbamate/thiocarbamate pesticide class. One study showed an increased risk of birth defects following parental exposure to fungicides during pregnancy, including an increased risk of anencephaly in children of mothers working in agriculture during the three months before conception and whose fathers also applied pesticides at work.[31]

These results provide some evidence that mancozeb is a reproductive toxicant. Mancozeb exposure may interfere with normal reproductive functioning of women persistently exposed (either occupationally or indirectly by spouse's occupation) and thereby may delay or complicate pregnancy. Very few population-based studies have examined the association between exposure to mancozeb and adverse reproductive or developmental outcomes.

Figure 1. Flow diagram of study selection process for systematic review.



Overview of Systematic Review Protocol

(Adapted from National Toxicology Program Office of Health Assessment and Translation approach for systematic review and evidence integration from literature-based health assessments)

1. Draft protocol strategy
    a. Discuss literature search strategy
    b. Determine criteria for selection of studies relevant to review question
    c. Discuss data abstraction components (risk of bias assessment and confidence in evidence)
2. Search strategy
    a. Decide on search criteria
    b. Search literature
    c. Read abstracts and select studies to include in review; obtain full paper
3. Data abstraction
    a. Develop and test data abstraction form
    b. Extract data & assess quality of study using OHAT criteria (BPA example)
    c. Assess confidence in studies
4. Translate confidence ratings into level of evidence for health effect

Table 1. *In vitro* studies (seven studies) examining exposure to Mancozeb and reproductive and developmental outcomes.

Source	Type of cell/tissue	Compound (Daily Dose)	Incubation	Outcomes	Confidence*
Lin & Garry, 2000[1]	MCF-7 breast cancer cell line	*Herbicides*: 2,4-D LV4 (0.1-10 ug/ml) 2,4-D Amine (1-10 ug/ml) 2,4-Dichlorophenoxyacetic acid, isooctyl ester (1-10 ug/ml) 2,4-Dichlorophenoxyacetic acid Roundup (1-10 ug/ml) Glyphosate (0.228-2.28 ug/ml) *Fungicides*: Triphenyltin acetate Mancozeb (reagent grade) (10-50 ug/ml) Manzate (commercial grade, 75% mancozeb) Tilt (reagent grade) Tilt (commercial grade, 42% propiconazole)	Test chemicals added within 2 to 4 hours of incubation and cultured for 72 hours in media	Three fungicides (triphenyltin, mancozeb,and Tilt) induced cytotoxicity over the concentration range tested (50–70% decrease in cell number at the highest concentration tested) Triphenyltin (4.1 ug/ml) and mancozeb (at 10 and 50 µg/ml) showed an apoptotic pattern of cell death between 24 and 48hrs. ETU induced apoptosis at a much higher concentration (1000 ug/ml).	+++
Bisson et al. 2002[2]	Adrenocortical cells of rainbow trout	Atrazine (0.0005, 0.05, 5, 500 uM) Diazinon (0.05, 0.5, 5, 50, and 500 uM) Endosulfan (0.05, 0.5, 5, 50, and 500 uM) Mancozeb (0.5, 5, 50, 500 and 5,000 uM)	60 minutes	Mancozeb exposed cells showed significant effects on cortisol secretion and on viability. • Significant impairment of cortisol secretion and no significant reduction of viability at a concentration of 500 uM. • Significant reduction of cortisol secretion (no reduction in viability reported) at 50 uM of mancozeb. • Mancozeb had the highest LC50/EC50 ratio*. *The ratio LC50/ EC50 for mancozeb was 16—a concentration about 16-fold higher was required to kill 50% of cells than the concentration required to impair 50% of cortisol secretion.	++++

Fejes et al. 2002[3]	288 chicken embryos	80% mancozeb containing formulation (Dithane M-45) Copper-sulfate (0.1% applied concentration)	19 days	Mancozeb did not increase embroytoxicity or result in an increased incidence in developmental abnormalities. Increased embryomortality was observed in embryos exposed to both mancozeb and copper-sulfate.	++
Greenlee et al. 2004[4]	Mice embryos	Several agrochemicals were tested at low-doses in the study. Mancozeb (.003 ug/mL)	96 hours	Mancozeb induced developmental injury in pre-implementation embryos Fungicide mixture (including mancozeb) reduced development to blastocyst and increased apoptosis	+++
Calviello et al. 2006[5]	Wistar rats: 1) RAT-1 fibroblasts 2) Peripheral blood mononucleated cells (PBMC)	Mancozeb and Zineb (concentrations ranging from 125 to 500 ng/ml)	1 hour and 4 hours	*At 1 hour* of mancozeb exposure (500ng/mL): • Cells produced dose-dependent induction of single strand breaks in RAT-1 • Increased levels of markers of DNA oxidation and reactive oxygen species in RAT-1 • PBMC less responsive than RAT-1 to oxidative insult *After 4 hours* of exposure, results showed dose-dependent: • Oxidative effect and prooxidant action of Mancozeb • Apoptosis in PBMC and RAT-1 cells	++++
Paro et al. 2012[6]	Mouse granulosa cells and human granulosa cells from women undergoing assisted reproductive therapy	Mancozeb (increasing concentrations (0.001-1 ug/ml)	1, 24, and 36 hours	Mouse granulosa cells exposed to mancozeb showed time- and dose-dependent changes in morphology, acquired ability to migrate, decreased level of p53, and no change in apoptosis Human granulosa cells showed dose-dependent changes in morphology and reduction in p53 expression post-mancozeb exposure.	++++
Kjeldsen et al. 2013[7]	human breast carcinoma MVLN cells and	14 pesticides were tested Mancozeb concentrations tested singly:	48 hours	When tested as a single compound, mancozeb exposure showed concentration-dependent antiandrogenic	++++

	hamster ovary CHO-K1 cells	LOEC (M): 1 x 10^{-5} MOEC (M): 1 x 10^{-5} % of Solvent control (0.02% DMSO): 45		effects on agonist-induced AR transactivity. At the lowest tested dose inducing the maximum effect (MOEC), mancozeb showed the highest inhibitory effect in the form of an agonist-reduced response.	

*Confidence ratings in body of evidence ratings: *High confidence* (++++) in the association between exposure to mancozeb and the outcome. The true effect is highly likely to be reflected by the apparent relationship; Moderate confidence (+++) in the association between exposure to mancozeb and the outcome. The true effect is may be reflected in the apparent relationship; *Low confidence* (++)in the association between exposure to mancozeb and the outcome. The true effect is likely to be different than the apparent relationship; and *Very low confidence* (+) in the association between exposure to mancozeb and the outcome. The true effect is highly likely to be different than the apparent relationship.

Table 2. *In vivo* studies (fifteen studies) examining exposure to Mancozeb and reproductive and developmental outcomes.

Source	Experimental Animal	n	Compound (route)	Daily Dose	Duration	Outcomes	Rating*
Khera 1976[8]	Nulliparous adult Wistar female rats & New Zealand White rabbits	209 rats 33 rabbits	Mancozeb (oral)	0, 5, 10, 20, 40, or 80 mg/kg/day	Rats: *Group I*: 21-42 days BG and GD 1-15; *Group II*: 6-15 GD and *Group III*: GD 7-20 Rabbits: 30 GDs	*Prenatal survival in rats.* Numbers of corpora lutea, live fetuses, and fetal death at all doses were similar to controls in all the three experiments. Doses of 80 mg/kg in *Group 2* and 40 mg/kg in *Groups 1* and *3* reduced the mean fetal weight as compared to matching control groups. ETU at the maximum tolerated doses administered before and during gestation had no significant effect on any reproductive parameter. *Teratogenicity in rats.* ETU was teratogenic in rats at doses that produced no apparent maternal toxicity or fetal lethality. Significant morphological fetal abnormalities were observed (including jaw, tongue, eyelids, short or absent tail malformations); fetal brain appeared to be the most commonly affected organ.	++++

						Prenatal survival in rabbits. Trend observed for (i) increased frequency of resorption sites (including dead fetuses), and (ii) reduced brain weigh at 80 mg/kg. *Teratogenicity in rabbits.* No skeletal abnormalities observed in animals treated with mancozeb. Rabbit fetal brain was insensitive to teratogenic effects at any dose level.	
Larsson et al. 1976[9]	Three-month-old NMRI mice and virgin female Sprague-Dawley rats	56 mice 56 rats	Maneb, Mancozeb, and Propineb (oral)	Mice received 400, 770, or 1420 mg/kg Maneb 380, 730, or 1330 mg/kg Mancozeb 400, 760, or 2300 mg/kg Propineb	18 GDs	*Mouse*: No adverse maternal effects or fetal effects were observed for the different dosages of maneb or mancozeb. Fetal weight was not reduced in the experimental group compared to control groups except in the fetuses from mothers treated with the lowest dose of mancozeb on day 9. *Rat*: Gross malformations occurred in all surviving fetuses after treatment with the medium and high dosages of maneb and in 25% of those exposed to the highest dose of mancozeb. Superficial hemorrhages were found in the 2 highest mancozeb dosage groups. Frequency of internal malformations was extremely high compared with that of external malformations for highest dose of mancozeb.	+++
Khan & Sinha, 1996[19]	males of Swiss albino mice	56	Endosulfan, Phosphamidon, and Mancozeb (oral) Vitamin C (injection)	Pesticides: 3, 6 or 1000 mg/kg/day Vitamin C:	35 days	Significant decreases in mean sperm counts in each of the three pesticide-treated groups compared to controls. All three categories of sperm head	++++

				10 (lower dose), 20 (middle dose) and 40 mg/kg body wt/day (higher dose)		abnormalities increased significantly (compared to control) in the groups treated with each of the three pesticides. Sperm with abnormal head size were most common in the mancozeb-treated group.	
Kacker et al. 1997[20]	Male albino rats	200	Mancozeb in peanut oil (oral)	500, 1,000, and 1,500 mg/kg/day	30, 60, 90, 180, and 360 days	Mancozeb at all doses produced signs of poisoning (diarrhea, dyspnea, salivation, nasal bleeding, hind limb paralysis). Mortality was more pronounced during days 0-90 as compared to days 90-360. Higher doses (1,000 and 1,500 mg/kg/d) over a period of 180 and 360 days produced a slight increase in relative weight of testes and a decrease in epididymis. Testes of rats dosed with mancozeb (1,500 mg/kg/d) produced time dependent pathological changes in seminiferous tubules and showed damaged epithelial cells in the tubules of epididymis with declines in sperm count. Increased activity of LDH, ALP and decreased activity of ACP and SDH in testes and epididymis.	++++
Roperto & Galati 1998[32]	nonmigratory pigeons	10	mixture of 30% mancozeb and 15% copper oxychloride	--	Longterm exposure over years	**Pigeons living in control environment:** No apparent optical and ultrastructural lesions were found in the trachea or lungs via histopathology, scanning EM, or x-ray. The metallic components of the fungicidal mixture (Cu and Zn) were not detected in the lungs via spectrometer. **Pigeons living in the exposed environment:** Severity of the histopathological lesions	+++

						was dependent on age. Intimal proliferation of the intra-parabronchial arteries and scattered foci of fibrosis were also evident as was deposition of Zn and Cu particles throughout lungs.	
Castro et al. 1999[10]	Wister rats	120	Mancozeb (mixed into diet)	0, 2000 or 3.000 ppm	*Group A*: GD 1-6, *Group B*: GD 6-15,	Mancozeb exposure did not interfere with estrous cycle in pregnant females, maternal weight gain, nor percentage of birth viability of pups. Pups of mancozeb exposed dams experienced delays in physical development. Prenatal mancozeb exposure also resulted in developmental motor alterations in pups.	+++
Mahadevaswami et al. 2000[13]	Female Wistar virgin rats	36	Mancozeb (oral)	500, 600, 700, and 800 mg/kg/day	15 BG days	Treatment with 700 and 800 mg/kg/day mancozeb showed a decrease in ovarian hypertrophy. No significant change in the number of estrous cycles and duration of each phase of the estrous cycle with 500 mg/kg/day mancozeb. Decreased number of estrous cycles, duration of proestrus, estrus, and metestrus with significant increase in the duration of the diestrus phase with > 600 mg/kg/day mancozeb treatment. Decreased number of healthy follicles with increase in the number of atretic follicles at higher doses of mancozeb. No significant changes in the body and organ weight with any dose level of mancozeb.	++++

						Treatment with 600, 700, and 800 mg/kg/day mancozeb showed a significant decrease in the levels of protein, glycogen, total lipid, phospholipid, and neutral lipid in the liver, uterus, and ovary, with the exception of liver total lipid and uterine glycogen. Mancozeb treatment reduced the number of healthy follicles with a concomitant increase in the number of atretic follicles.	
Baligar & Kaliwal 2001[12]	Wister rats (virgin)	40	Mancozeb (oral)	500, 600, 700 and 800 mg/kg/d	30 days	Significant decrease in the number of estrous cycle, duration of proestrus, estrus and metestrus with concomitant increase in duration of diestrus in all mancozeb treated groups. Significant decrease in the number of healthy follicles and increase in atretic follicles in all mancozeb treated groups. Reduced number of corpora lutea and size of ovary in high doses of mancozeb treated rats. Significant increase in thyroid weight in all mancozeb treated rats except in 500 mg/kg/d dosage group.	+++
Ksheerasagar & Kaliwal 2003[21]	Male Swiss albino mice		Mancozeb (oral)	800 mg/kg/day	5, 10, 20 and 30 days	Weight of testes decreased significantly with increasing durations of pesticide treatment in mice. Increasing durations of mancozeb resulted in a significant decrease in the weight of liver, kidneys and spleen. Significant increase in the thyroid and thymus weight in duration dependent	++++

						manner.	
Baligar & Kaliwal 2004[11]	Female virgin albino rats	70	Mancozeb (75% wettable powder) dissolved in olive oil (oral)	700 mg/kg/d	5, 10, 20 or 30 BG days	Mancozeb treatment for 1) *5 days*- significant increase in the duration of diestrus and decrease in state II and total number of healthy follicles. 2) *10 days*- significant increase in the number of estrous cycles and duration of estrous, with increase in diestrus; decrease in stages I, II, and IV and in total number of healthy follicles. 3) *20 and 30 days*-number of estrous cycles and duration of proestrus, estrus, and metestrus were significantly decreased, with increase in duration of diestrus; increase in atretic follicles and thyroid weight	+++
Miranda-Contreras et al. 2005[16]	Pregnant NMRI mice	160	Paraquat [PQ] and Mancozeb [MZ] (injection)	*Group I*: saline, *Group II*: PQ (10 mg/kg), *Group III*: MZ (30 mg/kg) or *Group IV*: the combination of PQ and MZ (10 mg/kg PQ + 30 mg/kg MZ)	Pregnant mice: GD 1-30 Dams: PD 12 and PD 20	*Body Weight* Second postnatal week: Significant reductions in body weight were found in mice exposed to PQ + MZ After 1 month: • marked decline (27%) in body weight as compared with the control group (permanent effects of prenatal exposure to PQ + MZ on the physical growth of the animals). • PQ-exposed group showed significant declines 21% and 17% for the MZ-exposed animals Pups showed significant reductions in excitatory amino acid neurotransmitters during cerebellar cortex development of prenatally exposed mice to chronic doses of MZ and PQ + MZ.	++++

						In MZ-exposed important variations in the developmental variation in Gly levels compared to control were registered: a 36% reduction at P3, a 150% increment at P15 and a 43% fall at P30. MZ exposed groups showed general trend of reduction in inhibitory neurotransmitters during cerebellar cortex development. *Locomotor activity* Mice prenatally exposed to MZ were more active by 3.1 fold compared to controls; at P30 locomotor activity was significantly lower than controls for all three experimental groups. Prenatal exposure to either PQ or MZ or the combination of both may lead to alterations to the chronology and magnitude of synaptic transmission in developing mouse cerebellar cortex.	
Rossi et al. 2006[14]	Swiss CD1 female mice	25	Mancozeb in sesame oil (oral)	50 and 500 mg/kg	GD 2 to PD 20	No significant difference in mean number of oocytes from F1 generation treated with either low (50 mg/kg) or high (500 mg/kg) doses of mancozeb compared to controls. Production of MII-arrested oocytes in F1 generation was slightly, although significantly, reduced in mice exposed to high doses (500 mg/kg) of man-cozeb; exposure to low doses (50mg/kg) was ineffective. 82% of the oocytes derived from control or low dose treatment groups formed male and female pronuclei	+++

						71% of oocytes derived from the high dose treatment	
Axelstad et al. 2011[15]	Wister rats	64 (litters)	Mancozeb in corn oil (oral)	0, 50, 100, or 150 mg/kg/d	GD 7 to PD16	Doses of 150 mg/kg bw/day and above caused toxic effects in the dosed dams (e.g., severe weight loss and transient paralysis of the hind limbs) T4 levels showed a dose-dependent and significant decline in dams from all three dose groups on gestational day 15 (offspring T4 levels, thyroid weights and histology were unaffected on postnatal day 16).	++++
Hass et al. 2012[18]	Time-mated nulliparous, young adult female Wistar rats	198	procymidone, epoxiconazole, tebuconazole, mancozeb, and prochloraz (gavage)	procymidone: 12.5 and 50 mg/kg/day; epoxiconazol: 3.75 and 15 mg/kg/day; tebuconazole: 12.5 and 50 mg/kg/day; mancozeb: 6.25 and 25 mg/kg/day; and prochloraz: 8.75 and 35 mg/kg/day	GD 7-21, PN 1-16	No significant effect on gestation length, maternal or pup body weight gain for rats exposed to mancozeb. Gestation length was significantly increased in the two highest mixture groups compared to controls. Nipple retention in male offspring was significantly increased in the groups exposed to the mixture and in the groups exposed to the high dose of mancozeb, tebuconazole and both doses of procymidone. One male in each of the groups dosed with mancozeb showed malformation of the seminal vesicle. All pesticides except for mancozeb exhibited AR antagonism in vitro. Combined developmental exposure to endocrine disrupting pesticides at dose levels below NOAELs for the single pesticides caused adverse effects on male sexual development and gestation length.	++++
Jacobsen et al.	Time-mated	198	procymidone,	Procymidone:	Dams were	One animal from each of the mancozeb	++++

2012[17]	nulliparous, young adult female Wistar rats		epoxiconazole, tebuconazole, mancozeb, and prochloraz (gavage)	12.5 and 50 mg/kg/day; epoxiconazole : 3.75 and 15 mg/kg/day; tebuconazole: 12.5 and 50 mg/kg/day; mancozeb: 6.25 and 25 mg/kg/day; prochloraz: 8.75 and 35 mg/kg/day mixture: 0, 14.6 (8.3% of NOAEL), 29.2 (17% of NOAEL) or 43.8 (25% of NOAEL) mg/kg/day of the mixture of the 5 pesticides	dosed daily by gavage, from GD 7 to pup day (PD) 16	groups and one animal from the low dose mixture group had more than double thyroid weight com- pared to controls. Significant increase in the number of animals with follicles dominated by columnar to cuboidal epithelium was seen in mancozeb exposed animals compared to the control group (observed in 4 of 9 animals from the high dose mancozeb group compared to 1 of 17 control animals). Significantly higher activity levels were observed in females from the high dose mancozeb group. **Note: Lowest dose of each pesticide was similar to the dose included in the highest mixture dose and then highest dose of the single pesticides was 4 times higher, corresponding to 25% of NOAEL	

BG=Before Gestation, GD=Gestation day, PD=Postnatal day, mg/kg/d= mg/kg body weight/day, NF=not found

*Confidence ratings in body of evidence ratings: *High confidence* (++++) in the association between exposure to mancozeb and the outcome. The true effect is highly likely to be reflected by the apparent relationship; Moderate confidence (+++) in the association between exposure to mancozeb and the outcome. The true effect is may be reflected in the apparent relationship; *Low confidence* (++)in the association between exposure to mancozeb and the outcome. The true effect is likely to be different than the apparent relationship; and *Very low confidence* (+) in the association between exposure to mancozeb and the outcome. The true effect is highly likely to be different than the apparent relationship.

Table 3. Epidemiologic studies (fourteen studies) examining exposure to Mancozeb and reproductive and developmental outcomes.
[¶]Design—Co: Cohort; RCo: Retrospective Cohort; CaCo: Case-Control; CrSe: Cross-sectional; and CaS: Case Series/Case report.

Source	Design[¶]	Population	Mancozeb Assessment	Outcome	Confidence*
Arbuckle et al., 2001[28]	RCo	Farm couples in the Ontario Family Health Study (N=2,110 women)	Pooled pesticide exposure information from the farm operator, husband, and wife to construct a history of monthly agricultural and residential pesticide use. Four pesticide classes: 1) Herbicide, 2) Insecticide, 3) Fungicide and 4) Miscellaneous. **Note: Mancozeb was not directly mentioned or measured in the study	Preconception exposure to thiocarbamates (OR = 1.8; 95% CI, 1.1–3.0), glyphosate (OR = 1.7; 95% CI, 1.0–2.9), fungicides (OR = 1.4; 95% CI, 0.9–2.1), and the miscellaneous class of pesticides (OR = 1.5; 95% CI, 1.0–2.4) were associated with elevated risks of late spontaneous abortions (12-19 weeks). Exposure to both fungicides and herbicides before conception doubled the risk relative of spontaneous abortions compared to woman exposed only to fungicides (OR = 2.0; 95% CI, 1.1–3.5). Exposure to fungicides doubled the risk of having a spontaneous abortion compared to those not exposed (OR = 2.4; 95% CI, 1.0–5.9) in older women (age 35+). No increased risk was observed in younger women.	+++
Bell et al., 2001[30]	CaCo	*Cases*=319 neonatal deaths within 24 hours of birth and *controls*= 611 normal live births in 10 California counties in 1984.	County maps to locate the township, range, and section for each maternal address (maternal TRS) was determined by using the public land survey system. Pesticide exposure was determined by linking the maternal TRS to the township, range, and section of each pesticide application located in the California Pesticide Use Report database. Five categories were selected for analysis: phosphates, carbamates, pyrethroids, halogenated hydro-carbons, and endocrine disruptors. *Mancozeb was assigned to the Carbamates/thiocarbamates class*.	Pesticide classes were not strongly associated with fetal death, regardless of trimester of exposure. Weakly elevated risks were observed for applications of halogenated hydrocarbons, carbamates/thiocarbamates, estrogenic pesticides, and carbamate acetylcholinesterase inhibitors during the second trimester, with HRs of 1.3 (95 percent confidence interval (CI): 1.0, 1.8), 1.3 (95 percent CI: 1.0, 1.8), 1.4 (95 percent CI: 0.8, 2.5), and 1.3 (95 percent CI: 1.0,1.8), respectively. Modestly increased risk of fetal death was observed when exposure to halogenated hydrocarbons occurred during the fourth and fifth months of gestation, with HRs of 1.4 (95 percent CI: 1.0, 2.0) for both months. Results were similar for exposure to carbamates and carbamate inhibitors during the third and fourth months of gestation. Slightly elevated HRs were observed for exposure to carbamates during the first and second trimesters of gestation, the monthly analysis showed that risk increased only for exposure during the third and fourth months of gestation.	++
Colosio et al., 2002[22]	CaCo (pilot)	Italian vineyard workers (N=13)	First urine spot sample collected in the morning pre-	Urinary ETU was significantly increased at the end of shift (2.5, 0.5–95.2 mg/g creatinine) in workers compared with baseline	++

		and controls (N=13)	mancozeb application. Dermal exposure assessment captured morning in which mancozeb mixture was prepared and applied by workers (pads were placed on clothes and skin) * assessment of the exposure was performed in the absence of previous applications of mancozeb and/or other pesticides	levels. End-shift urinary ETU levels were higher in operators using open tractors (n=7) than in those using closed tractors (n=5) (16.2 vs. 2.4 mg/g creatinine), but not statistically significant. End-shift urinary ETU was positively correlated with dermal exposure to mancozeb determined both over the clothes and on the skin.	
Garry et al., 2002[29]	CrSe	1,070 licensed pesticide applicators (males), 851 were married or had a marriage-like relationship, residing in five counties in Red River Valley, Minnesota, USA	*Pesticide use* Each certified pesticide applicator was initially inter-viewed by phone to obtain current and past pesticide use with specific attention to product name and the number of days per year applied. 6 months follow-up questionnaire to document common pesticide use by pesticide class, acreage treated, type of crop, and use of personal protective gear. Overlap between the two questionnaires was intentional to validate use of pesticides by class (herbicides, insecticides, fumigants, and fungicides).	<u>Male Pesticide Use, Fetal Loss, and Miscarriages</u> *Sample: 540 farm families with children, where the applicator was the biologic father or whose spouse had been pregnant in the relationship, participated and gave detailed pesticide use data. Among couples who had children or who had been pregnant, one in four women had a miscarriage. Spouses of pesticide applicators who reported use of herbicides, insecticides, and fungicides had more miscarriages than any other pesticide application group. <u>Specific Fungicide Use</u> The unadjusted odds ratio for specific use of organotin (OR = 1.55; CI 1.01–2.37) and/or use of EBDC (ethylene bisdithiocarbamate) fungicides (OR = 1.77; CI 1.11–2.83) such as mancozeb demonstrates a significantly increased frequency of fetal loss compared to nonuse of fungicides by applicators. <u>Maternal Pesticide Exposure</u> *Sample: Only 36 of 379 women respondents applied or mixed pesticides. Personal use of pesticides, (e.g., mixing, loading and pesticide applications) by the female spouse, was a significant risk factor for fetal loss (fetal loss/pregnancy OR = 1.81; CI 1.04–3.12). 30% had a fetal loss compared to 21% of those women who did not mix or apply pesticides. The number of fetal losses per pregnancy is significantly elevated	+++

				in those who did mix or apply pesticides (15% vs. 8.9%). Miscarriage and Season # of first trimester miscarriages in spring was significantly elevated compared to all other seasons. Spouses of applicators who applied fungicides and other pesticides showed no peak in the miscarriage frequency in spring. Frequency of first-trimester miscarriages was significantly higher in summer, fall, and winter combined for the spouses of applicators of fungicides compared to applicator families whose male spouse did not apply fungicides. Significant shift in the sex ratio of children born to the spouse of appliers who applied fungicides. • 25% reduction in male births in the children of applicators who applied herbicides, insecticides, and fungicides or among those applicators who applied mancozeb and/or triphenyltin fungicides was significant.	
Garry et al., 2002[33]	CrSe	Families of licensed pesticide applicators in Red River Valley, Minnesota, USA (N=536 couples)	Phone survey on current and past pesticide use in agriculture with 6 month follow-up interview to validate pesticide class. *Class of pesticide* **Herbicides** Chlorophenoxy Oxyphenoxy Sulfonylurea Carbanilate Bromophenol Benzothiazole Nitroaniline **Insecticides** Organophosphate Synthetic pyrethroids Carbamate **Fungicides** Organotin Ethylene bisdithiocarbamate Triazole	In families whose male partner did not apply fungicides, increased frequency of males born compared to men who applied fungicide products. Increased frequency of male children born with birth defects than female children (M/F sex ratio = 1.8) when no fungicides were applied. If fungicides were applied by the male partner, far fewer male children with birth defects were born (M/F sex ratio = 0.57, p = 0.02). No difference observed in birth rate for families of fungicide applicators compared to applicators of herbicides and other products (2.85 vs. 2.80 children per family).	++

			Fumigants Phosphide Other		
Sallmen et al., 2003[34]	Co	Finnish greenhouse worker: employers (N=419) and employees (N=14,733)	Exposure to pesticides was assessed on the basis of questionnaire information and data gathered from the employers	Reduced fecundability in exposed greenhouse workers not wearing proper personal protection equipment (FDR 0.67 95%CI: 0.33-1.35). 0.92 (85%CI: 0.45-1.88) and 0.77 (95%CI 0.46-1.29) for high exposure, moderate, and low exposure groups compared to unexposed greenhouse workers. Fertility was comparable between exposed workers who used PPE and unexposed greenhouse workers	++
Farr et al., 2004[25]	CrSe	Women living on farms in Iowa and North Carolina (N=3,103)	pesticide exposure questions assessed lifetime use of any pesticides and hormonally active pesticides women were asked, in their lifetime, had they ever mixed or applied any of 50 different pesticides	Carbamate pesticides use associated with increased odds of long menstrual cycles (OR = 2.1) and decreased odds of irregular cycles (OR = 0.38). Women who used mancozeb or maneb had four times the odds of experiencing long cycles and two times the odds of missed periods as women who had never used pesticides. No other outcomes were associated with the use of mancozeb or maneb. A subanalysis excluding female licensed applicators and spouses who had not likely used hormonally active pesticides in the last year results showed elevated odds of long cycles, missed periods, and intermenstrual bleeding among women exposed to lindane, atrazine, or mancozeb or maneb compared to unexposed women. *Models adjusted for age, body mass index, and current smoking status.	++
Corsini et al., 2005[24]	CaCo	13 Vineyard workers and 13 matched healthy controls in Northern Italy	*Workers*: urine and blood samples were collected before the beginning of the spraying season, prior to any exposure to pesticides, and at the end of the work shift of the last day of Mancozeb application. *Controls*: urine and blood samples were collected only	*Urinary ETU metabolite results:* Significant increase in urine ETU concentration among applicators in post-exposure compared to baseline values and controls samples. No difference observed in baseline levels of urine ETU in workers or controls. *Blood cell counts:* Significant increase in CD19+ cells and decrease in CD25+ cells	+++

			once in the morning at the same time of the year of workers.	were detected in follow-up blood samples compared to controls. Significant reduction in eosinophil and serum IgE in workers post-exposure. Significant increase in the proliferative response to phorbol myristate acetate plus ionomycin (PMA + ionomycin) was observed in the post-exposure group compared to controls and baseline. Significant decrease observed in LPS-induced TNF-a release post-exposure in workers.	
Idrovo et al., 2005[27]	CrSe	Female flower production workers in Bogota, Colombia (N=2,085).	Exposure matrix constructed based on work in flowers and time in agriculture; exposure was categorized as low, medium, and high. *Exposure to pesticides is year-round and fungicides (especially dithiocarbamates such as propineb and mancozeb) are more commonly used for 1 out of 5 industries. An estimated 5.7% of industries used mancozeb.	A trend in longer time to pregnancy (TTP) was observed in women engaged in any type of waged work. Positive dose-response relationship between duration of work in flower production and TTP.	++
Nordby et al., 2005[35]	RCo	National registers in Norway, identifying female (n=105 403) and male farmers (n= 131,243) and their children (n=300, 805) born in 1925-1971.	Data on farm production and fungal forecasts (humid and temperate weather conditions) in 1973–1990, obtained from agricultural censuses and meteorological measurement stations, respectively, served as the mancozeb exposure indicators.	Neural tube defects (131 cases, prevalence 12.8/10 000 births) was moderately associated with potato cultivation (PR 1.6, 95% CI 1.1–2.3) and paternal work of >500 hours/year (PR 1.6, 95% CI 1.1–2.5). 319 thyroid cancer cases were identified: 1) 141 in female farmers (incidence 10.2/100 000 person years), 2) 79 in male farmers (incidence 3.2/100 000 person-years), 3) 99 in offspring (female and male incidence 3.4 and 0.6/100 000 person-years). Mancozeb exposure was not associated with thyroid cancer.	+++
Lascana et al., 2006[31]	CaCo (paired)	151 cases of anencephaly of >	Questionnaire obtaining information on:	Children of mothers who worked in agriculture in the ARP (acute risk period), that is periconceptional period, had a greater risk of	+++

		20 wks' gestation 151 controls born in three Mexican states	1) lifetime occupational activities (start/end dates of job); 2) time involved in agricultural work, application of pesticides, and/or other activities with potential exposure to these substances (manufacture, formulation, sale, or distribution of pesticides; work in fruit or vegetable warehouses; pest control work or gardening); 3) direct handling of pesticides and time periods involved. . Work in agriculture was categorized as nonagricultural, agricultural, and applicators.	anencephaly (OR = 4.57, 95% CI 1.05 to 19.96). Risk of fathers having a child with anencephaly was greater in those who applied pesticides irrespective of whether it was done in the periconceptonal or the non-acute risk period (OR = 2.50, 95%CI 0.73 to 8.64; and OR= 2.03, 95% CI 0.58 to 7.08, respectively). Pesticides most used by the parents of the cases in decreasing order of frequency were: permethrin (pyrethroid), methamidophos (organophosphate), methyl parathion (organophosphate), atrazine (triazine), 2,4-dichlorophenoxyacetic acid (chlorinated phenoxy), chlorpyrifos (organophosphate), mancozeb (dithiocarbamate), picloram (pyridine), dimethoate (organophosphate), and carbofuran (carbamate); pesticides most used by the parents of the controls in decreasing order of frequency were: methyl parathion (organophosphate), methamidophos (organophosphate), 2,4-dichloro- phenoxyacetic acid (chlorinated phenoxy), chlorpyrifos (organophosphate), and monocrotophos (organophosphate).	
Rull et al., 2006[26]	CaCo	Infants with neural tube defects (NTDs) (n=928) and normal formed controls (n=1263) delivered in California between 1987 and 1991	Potential exposures to specific restricted-use agricultural pesticides were evaluated by using a geographic metric based on linking pesticide-use reports from the California Department of Pesticide Regulation with land-use survey maps of crops from the Public Land Survey System. A physiochemical category for Dithiocarbamate and pesticide category for Mancozeb were included in the analysis. Mancozeb was classified as an endocrine disruptor.	Effect estimates for mancozeb applied within 1,000 meters of maternal residences on neural tube defects, California, 1987-1991 Cases: 6/731 Controls: 11/940 *Conventional logistic regression*: 1) Single-pesticide models: *OR*: 0.7 *95%CI*: 0.2, 1.9 2) Multiple-pesticide model: *OR*: 0.5 *95%CI*: 0.1, 2.3 *Hierarchical logistic regression*: 1) Multiple-pesticide model: *OR*: 0.5 *95%CI*: 0.2, 1.4 Exposure to any endocrine disruptors applied within 1,000 m of maternal residences resulted in an increased odds of 1.5 (95%CI: 1.2, 2.0) for any NTDs compared to no exposure of any endocrine disruptors. The effect of exposure to 2+ endocrine disruptors applied within 1,000 meters of maternal residences resulted in 2 times the odds of having a baby born with anencephaly compared to babies in the no	++

				exposure group (95%CI: 1.3, 3.0).	
Calvert et al., 2007[36]	CaS	Three mothers and their infants born with congenital abnormalities within 8 weeks of one another and whose mother worked for the same tomato grower	Exposure information was obtained from North Carolina Department of Agricultural and Consumer Services (NCDACS) and the Florida Department of Agriculture and Consumer Services (FLDACS), which obtained pesticide application and worker assignment records from the grower.	*Maternal Exposure History* *Case 1*: The period for limb development is 24–36 days after fertilization. During this period, this child's mother worked in violation of the REI for up to 4 days involving exposure to several pesticides, including mancozeb. *Case 2*: During gestational days 14–57, this child's mother worked in violation of the REI for up to 8 days. On seven of these days, the pesticides applied to the fields where the mother worked included methamidophos. In addition, on gestational days 7 and 10, the mother worked in fields when an REI was possibly in effect (mancozeb on both days, and abamectin and methylpyrrolidone on day 7). The mother has three other living children, none of whom are known to have birth defects. This mother also had one previous stillbirth but without obvious birth defects. *Case 3*: During gestational days 14–59, the mother worked in violation of REIs for up to 10 days. On eight of these days, the REI for methamidophos was in effect on some of the fields where the mother worked. Abamectin and methylpyrrolidone were applied to some of the fields on two other days, but the mother may have worked in those fields before the applications were made. The mother had two previous pregnancies. One pregnancy 3 years earlier involved a malformed fetus and ended in miscarriage. The mother could not recall her employment or whether she had any toxic exposures during that pregnancy. The other previous pregnancy resulted in a normal child. *Birth Outcomes* *Case 1*: This infant was born with tetraamelia (absence of all four limbs). *Case 2*: This infant was born with mild Pierre Robin syndrome (micrognathia, high arched palate, and mild persistent palatine rugae). *Case 3*: This infant had multiple severe malformations including cleft lip and palate, imperforate anus, solitary kidney, vertebral anomalies, dysplastic low-set ears, and ambiguous genitalia. Cases 1 and 2 were potentially exposed to mancozeb during the	+

				maximal sensitivity period. There is evidence to suggest that each mother was exposed to pesticides during the maximal sensitivity period for the organ system/structure that was affected. During the period of organogenesis (approximately 14–59 days after fertilization) when birth defects are most likely to occur, all three mothers unknowingly worked in tomato fields that were under an REI because the fields were recently treated with pesticides.	
Colosio et al., 2007[23]	CaCo	Ninety-three subjects entered the study: 48 vineyard workers intermittently exposed to mancozeb (14 from Pavia and 34 from Trento) and 45 control subjects (11 from Pavia and 34 from Trento) not exposed to known sources of pesticides in Northern Italy.	Recent exposure to mancozeb was assessed by measuring urinary ETU. Immunotoxic effects of mancozeb were studied using several blood cellular and serum parameters: complete and differential blood cell counts, lymphocyte subpopulations, aglycoproteins, erythrocyte sedimentation rate (ESR), complement 3 and 4 fractions (C3, C4), antinuclear (ANA), antismooth muscle (SMA) and antimitochondrial (AMA) autoantibodies, and serum immunoglobulins (IgG1, IgG4, IgM, IgA, and total and specific IgE to most common allergens). Three time points: 1) *T0 baseline*—before start of seasonal use of mancozeb; 2) *T30*-- exposure to mancozeb for 30 days; and 3) *T45*-- 40-45 days. Only time point 1 (T0) and 3 (T45) were obtained for controls.	*Urinary ETU* **T0**: A low but detectable amount of ETU observed in vineyard workers and controls at baseline. **T30**: Significant increase of urinary ETU in agricultural workers following exposure confirmed that absorption of mancozeb took place during application; Postexposure urinary ETU exceeded the reference value of 5 mg/g creatinine. *Blood* **T0**: Higher prevalence of cold or flu symptoms, lower percentage of monocytes, higher absolute count of T lymphocytes, CD4 and natural killer cells, and lower plasma levels of IgA and IgM in workers. These differences did not continue at T30 or T45. At T0, the agricultural workers showed lower plasma levels of IgA and IgM in comparison with controls. Within agricultural workers a significant increase of serum concentrations of IgA and IgM was observed after exposure. No biologically relevant differences were observed between groups (farmworker vs control) or at different times in other serum parameters. No statistically significant differences were observed in farmworker compared to control groups in the total or specific IgE levels.	+++

¶Design—Co: Cohort; RCo: Retrospective Cohort; CaCo: Case-Control; CrSe: Cross-sectional; and CaS: Case Series/Case report.
*Confidence ratings in body of evidence ratings: *High confidence* (++++) in the association between exposure to mancozeb and the outcome. The true effect is highly likely to be reflected by the apparent relationship; Moderate confidence (+++) in the association between exposure to mancozeb and the outcome. The true effect is may be reflected in the apparent relationship; *Low confidence* (++)in the association between exposure to mancozeb and the outcome. The true effect is likely to be different than the apparent relationship; and *Very low confidence* (+) in the association between exposure to mancozeb and the outcome. The true effect is highly likely to be different than the apparent relationship.

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